of which the mass spectrometer is the most important. Advances in this type of work will depend on advances in the instrumentation, particularly in the sensitivity of the mass spectrometer and on general advances in knowledge of food components under various circumstances.

See also: **II/Chromatography: Gas:** Detectors: Mass Spectrometry; Detectors: Selective. **III/Natural Products:** Liquid Chromatography. **Solid Phase Micro-Extraction:** Environmental Applications; Food Technology Applications. **Tobacco Volatiles: Gas Chromatography.**

Further Reading

There do not seem to be any modern texts which deal specifically with aroma analysis but the literature contains numerous references. The following is a partial list of papers published from 1997 to 1999 showing the wide variety of foodstuffs and drinks covered, ranging from wine, yogurt and tomato juice to strawberries and alligator meat. Although mass spectrometry is the main method of detection, other techniques are covered, together with a variety of methods for extraction prior to analysis.

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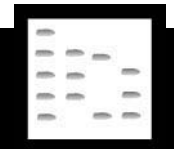
FUELS AND LUBRICANTS: SUPERCRITICAL FLUID CHROMATOGRAPHY



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Supercritical fluid chromatography (SFC) has a number of advantages over gas chromatography (GC) and high performance liquid chromatography (HPLC) for mixtures such as polyaromatic hydrocarbons (PAH). SFC operates with diffusivities that are more gas-like, viscosities that are lower than liquids, and densities that are more liquid-like. The resulting mass transfer coefficients lead to more rapid analysis in SFC than in HPLC. The diffusion and viscosity range available in SFC allows GC-like separations on capillary columns but at much lower temperatures.

SFC on Packed Columns

During the initial development of SFC, commercial HPLC columns were used. The length and internal diameter of SFC packed columns are constrained by the large pressure drop as compared to open tabular columns and high mass flow rates, making interfacing to GC detectors more difficult. Therefore, narrow-bore packed columns with diameters of 1–2 mm were frequently used because they can be installed in a capillary SFC instrument and are compatible with many GC detectors. Packed fused silica columns have significant advantages, allowing the use of a large variety of liquid chromatography (LC) stationary phases with GC-based detectors.

The separation of PAH standards has been used throughout the development of SFC to determine chromatographic efficiency and performance of the system. Various-size stationary-phase particles have been used (10, 5 and 3 μm particle diameter) and it has been shown that the particle size influences the efficiency of the columns and that SFC reduced analysis times considerably in comparison with HPLC.

The elution order of PAH in SFC can be varied by changing the operating temperature and/or the pressure. Also, the mobile-phase modifier used in SFC can significantly affect the retention behaviour of PAH; dramatic changes in retention together with different selectivities have been demonstrated. The addition of a modifier to the CO_2 mobile phase substantially reduces the retention times of the PAH. This effect is due to the intermolecular attraction between the modifier and the solute molecules and the subsequent increased solvating power. The successful separation of the 16 Environmental Protection Agency (EPA) target list of PAH using a single (15 cm × 4.6 mm) column packed with specially bonded C_{18} silica has been achieved in 6 min (**Figure 1**).

The analysis of PAH and their derivatives from particulate matter has been of recent interest due to possible human exposure of the highly toxic nitro-PAH compounds. Sandra *et al.* provides an interesting approach to the analysis of PAHs using semi-preparative SFC to separate the PAHs initially into the required types to analyse the peaks of interest, including nitro-PAH (**Figure 2**). A large amount of work has been carried out using supercritical fluid extraction (SFE) of particulates to extract the organic material followed by GC to characterize the extract.

Open Tubular Columns

The use of open tubular capillary columns in SFC for petroleum-derived mixtures was initiated by Lee *et al.* Capillary column SFC is mainly preferred because it provides the highest resolution. SFC can be carried out on capillary columns to achieve unique separations, especially for complex hydrocarbon mixtures

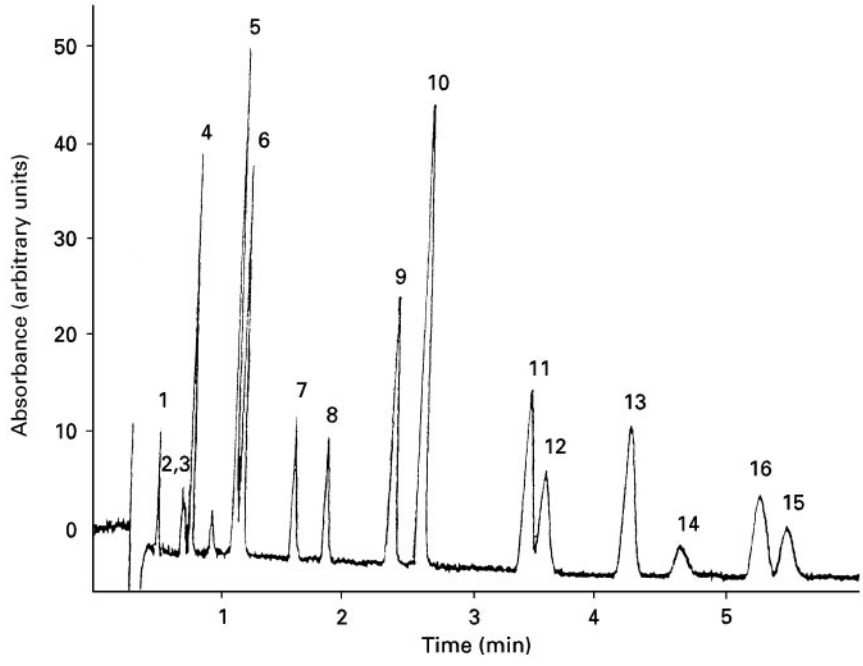

Figure 1 SFC of the EPA 16 priority PAH using 5 μm Waters Spherisorb PAH. Peak identification: 1, naphthalene; 2, acenaphthene; 3, acenaphthylene; 4, fluorene; 5, phenanthrene; 6, anthracene; 7, fluoranthene; 8, pyrene; 9, benzo[*a*]anthracene; 10, chrysene; 11, benzo[*b*]fluoranthene; 12, benzo[*k*]fluoranthene; 13, benzo[*a*]pyrene; 14, dibenzo[*a,h*]anthracene; 15, benzo[*ghi*]perylene; 16, 16 indeno [1,2,3-*cd*]pyrene. (Courtesy of the University of Leeds.)

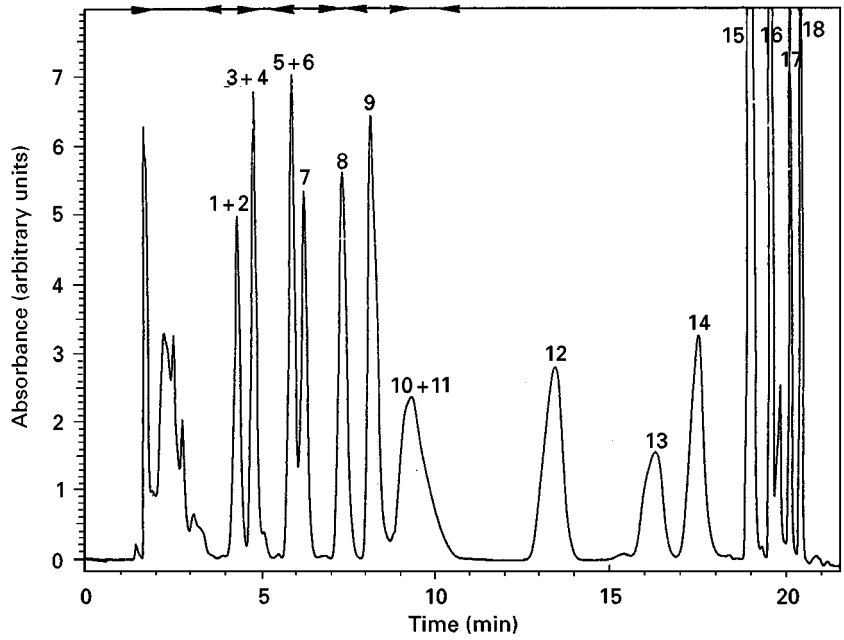

Figure 2 Semipreparative SFC separation of PAHs from particulate matter. Using a 25 cm × 4.6 mm i.d. silica column at 50°C and 200 bar, supercritical fluid flow rate 2 mL min^{-1}, a 1 : 1 methanol : acetonitrile modifier was used and programmed from 1% (up to 5 min) to 2.5% at 0.15% min^{-1}, and then 27.5% and 5% min^{-1}. With ultraviolet detection: 1.5–3.7 min 2–3-ring PAH; 4.4–5.8 min 4-ring PAH; 7.0–8.3 5-ring PAH; 9.0–10.9 6 ring PAH. (Reprinted with permission of Sandra *et al.* (1998) *J. Microcol. Sep.*, 10: 89.)

such as PAH. Capillary columns provide selectivity and high efficiency. Essentially, the same range of stationary phases that have been used for analysis in capillary GC have also been used in capillary SFC. These stationary phases include 100% methyl, 100% phenyl, 5% phenyl, 5% biphenyl and cyanopropyl siloxanes. An *n*-octylpolysiloxane coated stationary phase has been used to perform SFC on petroleum-derived vacuum residues. The increased alkyl content of the *n*-octyl phase over methyl phases provides an increase in van der Waals interactions of the stationary phase with solutes.

SFC separations on liquid crystal phases are based on the length-to-breadth (L/B) ratio and the planarity of the PAH, and they interact with the ordered rod-like structure of the liquid crystal phase. In addition, interactions such as dispersion, dipole and induced dipole interactions contribute to a good separation. Kithinji *et al.* optimized chromatographic conditions (pressure and temperature) to achieve better SFC separations of high-molecular-weight PAH on a capillary column coated with a smectic mesomorphic crystalline phase with simultaneous temperature and density programming. The separation of PAH isomers on a liquid crystalline polysiloxane stationary phase is shown in **Figure 3**. Many of these isomers cannot be resolved on any other stationary phase.

Raynor *et al.* used dual capillary column SFC with phases of different polarity and selectivity to perform simultaneous separation of a mixture of PAH isomers. The method not only reduces the development time by 50% but also provides two sets of retention data for identification of unknowns. The simultaneous separation of a mixture of three-, four-, five-ring, PAH isomers on biphenyl and smectic columns is shown in **Figure 4**.

However, unless column diameters are greatly reduced, open tubular column SFC cannot compete with conventional packed column SFC in terms of analysis time. Comparison of Figures 1 and 3 shows that separation of high-molecular-weight PAH can be achieved in less than 6 min with a conventional packed column, while more than an hour is required for an open tubular column.

Hydrocarbon Group-type Separations

Hydrocarbon group-type separations refer to the separation of alkanes, alkenes and aromatic compounds in petroleum feedstocks and products. LC is commonly used for this purpose but suffers from a lack of resolution, lack of a universal detector and long analysis time. GC has also been used but is limited to the analysis of light distillates due to the column temperatures required. Packed column SFC with CO_2 as the mobile phase has been used for the determination of saturates, olefins and aromatics in petroleum products boiling below 350°C. Separation is achieved

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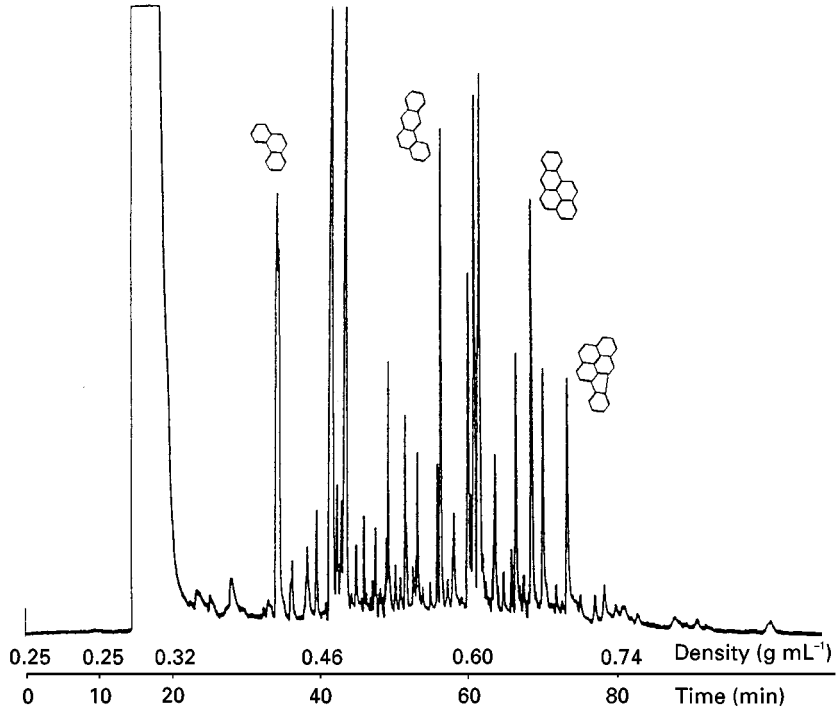

Figure 3 Chromatogram of total coal tar PAH obtained with linear density programming at a constant temperature of 110°C, 10 m × 50 μm, SB-smectic column. (Courtesy of the University of Leeds.)

using two different columns connected in series – a silica and a silver nitrate-impregnated silica column – the analysis takes only 4 min per sample. The effect of temperature, pressure and column stationary phase on the separation has also been studied. At a low temperature (35°C), the saturates are better separated from the monoaromatics.

Another advantage of using a low column temperature is the shorter retention times and hence shorter analysis time per sample. An increase in pressure also results in improved separation between saturates and aromatics. Separations using a combination of silica and cyano or amino columns or the combination of silica and 20% silver nitrate column are not as good as the separation obtained with a single 5 μm silica column at the same temperature.

The separation of aromatic types in middle distillates according to ring number has been studied. Two packed columns, a silica and an amino-modified silica, were used with a switching valve. The saturates are separated as a group from the aromatics on the silica column. The aromatics are then switched to the amino column where further separation into mono-, di- and polyaromatic types can be performed.

It has generally been found that the separation of saturates from olefins is incomplete when CO_2 is used as the mobile phase. This observation was thought to be due to the polarizability of CO_2 compared to the hydrocarbons used as solvents in LC. Another approach uses supercritical sulfur hexafluoride (SF_6) as the mobile phase. SF_6 provided less peak tailing and a shorter analysis time. Although SF_6 is reasonably compatible with the flame ionization detector (FID), the detector has to be protected against the decomposition product hydrogen fluoride, either by being gold-plated or by having a platinum jet and upper electrode.

A more accurate, reproducible and rapid SFC method to separate hydrocarbon mixtures by chemical class for samples ranging from C_4 to C_{40} uses a column-switching technique which allows interchange between a microbore (1 mm i.d.) silica gel column and a silver-ion loaded strong cation exchange silica gel microbore column, with 10% CO_2 in SF_6 as mobile phase. The silver-loaded cation exchange column gives the saturate and olefin separation, while temperature programming is used to elute the aromatic peaks. The only problem encountered with the use of silver-modified columns is that certain types of compounds can react with the silver ions, causing column instability.

The three-column system with column switching and backflushing can be used to separate saturates, aromatics and polar compounds in high-boiling residues. A multicolumn system for quantitative determination of crude oil and high-boiling fraction class separation has been developed. Three different col-

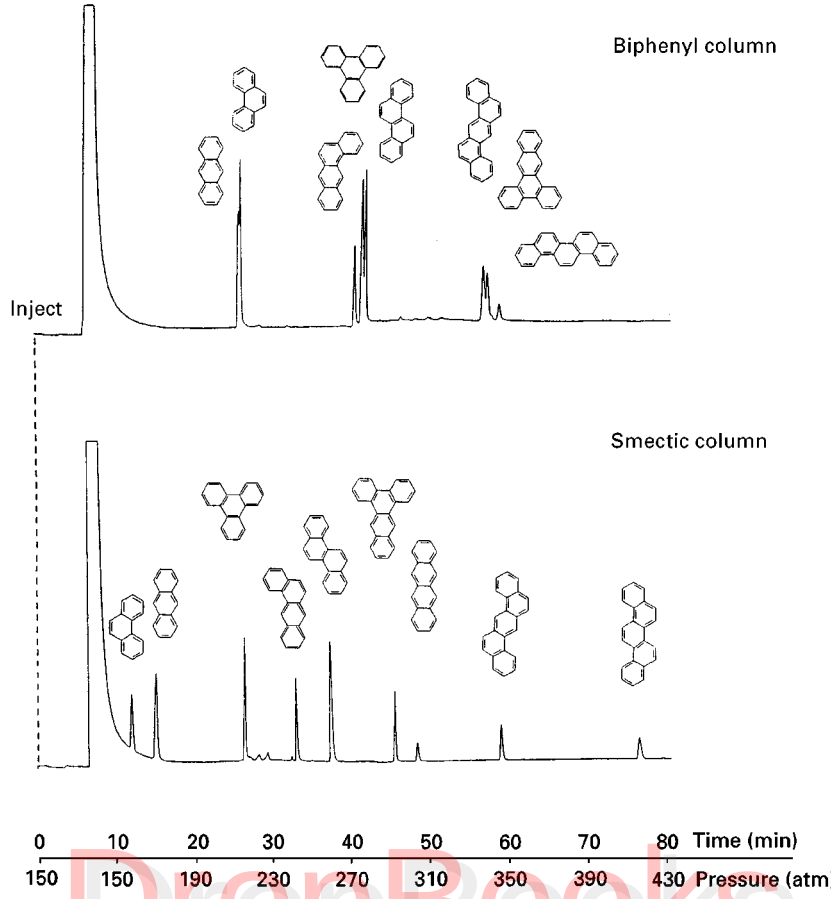

Figure 4 Simultaneous separation of three-, four- and five- ring PAH isomers on biphenyl and liquid crystalline columns. Conditions: CO_2 at 120°C; programmed from 150 bar (10 min) to 450 bar at 4 bar min^{-1}. (Courtesy of the University of Leeds.)

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umns are used – cyano, silica and silver-loaded silica – in order to separate saturate, aromatics and resins. The replacement of the silver-loaded silica column with a silver-loaded cation exchange column results in a more stable system. Similar effects have been observed when the silica column is replaced with a cyano column. The silica column prevents the aromatic components with strong π-interactions from entering the silver-loaded silica column and thus allows the aromatics to be backflushed from the silica column as a narrow band. The cyano column is used to trap the resins. Both carbon dioxide (CO_2) and nitrous oxide (N_2O) can be used as mobile phases; the latter provides better solubility of the resins and less tailing of the peaks.

A quantitative study of the determination of aromatics in jet and diesel fuels by SFC with FID used a single column (25 cm × 2.0 mm i.d. column packed with 5 μm Chromegasphere SI-60 silica), a CO_2 mobile phase under constant density and no temperature programming. It was found that the nonlinear response of the detector can be significantly improved by the addition of air as make-up gas (approximately 15 mL min^{-1}).

The use of a specially treated silica-based packed column to separate saturate and aromatic compounds in diesel fuels by SFC has been reported. Separations are achieved in less than 8 min, with good resolution (greater than 9) being achieved in the separation of one-, two- and three-ring aromatics.

In 1991, SFC was approved for the separation of saturate and aromatic hydrocarbons in diesel fuels. The American Society of Testing Materials (ASTM) method D5186 requires that temperatures in the range of 30–40°C be used for this separation. At these low temperatures, the separation of saturates and monoaromatics is easily achieved. However, low temperatures are not adequate for separation between the mono- and polyaromatics.

A packed capillary column has also been used with the same methodology, and showed that good separation can still be achieved at temperatures as high as 85°C. A further increase in pressure and temperature also results in apparently higher efficiencies for the

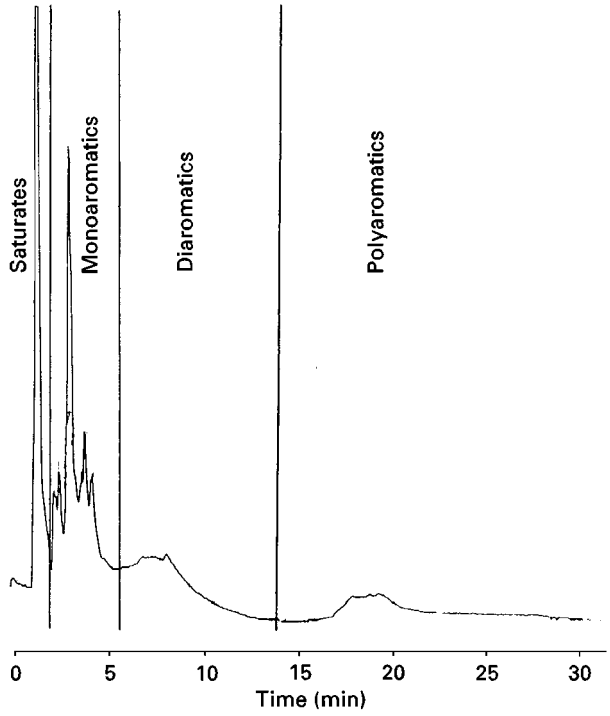

Figure 5 Chromatogram of base oil mixture containing 57.8% aromatic content. Operating conditions: column 1.3 × 250 μm i.d. packed with Waters Spherisorb S5W. (Courtesy of the University of Leeds.)

heavier aromatic peaks with reduced analysis time. However, the ASTM method requires a minimum resolution of 4 between the saturates and aromatics peaks. As the temperature is raised, the separation between the saturates and aromatics decreases. For highly complex mixtures, such as coal liquefaction recycle solvents, an optimized group-type separation procedure involves SFC on a 250 μm i.d. × 1.3 m long silica column operated at 80°C and 300 bar (**Figure 5**).

The separation of petroleum distillate into aliphatic and aromatic fractions has been achieved using a two-dimensional SFC-SFC system with a flow-switching interface. The columns used were a liquid crystal polysiloxane capillary column and a SB-Biphenyl-30 capillary. The use of a liquid crystal column in the second dimension to provide shape selectivity allows separation of various isomers, including chrysene, triphenylene, benz[*a*]anthracene and the benzofluoranthenes.

Simulated Distillation of Petroleum Compounds and Crude Oils

Distillation is the primary separation process in the petroleum used to characterize petroleum products before processing. Distillation data can be obtained by using true distillation techniques or analytical techniques which simulate the distillation process. Simulated distillation (SD) is a technique which is inexpensive and rapid compared to distillation techniques but does not yield fractions for further characterization. GC simulated distillation techniques require the use of special high-temperature columns, and the high temperatures (up to 400°C) may compromise the integrity of the sample. SFC uses much milder conditions (typically below 150°C) than those necessary for simulated distillation by GC and can be used for the characterization of heavy crude components, with boiling points up to 760°C. The effect of temperature and pressure on resolution and retention have been studied, and generally analysis is conducted by pressure programming while keeping the temperature constant, although simultaneous temperature and pressure programming has been successfully used. A relatively nonpolar, 5% phenyl–95% dimethylpolysiloxane (DB5) phase is used. Such columns are very stable at SFC temperatures and, with the use of an integral restrictor installed at the end of the column, good results are obtained.

Linear density programming of the supercritical mobile phase provides a more nearly linear relationship between the elution pressure and homologous series boiling point. The use of open tubular columns eliminates the pressure drop effects which are common in packed columns. If an *n*-octylpolysiloxane stationary phase is used, the discrimination between aliphatic versus aromatic boiling points is minimized.

While SFC with open tubular columns seem to be well suited to SD, low sample capacity and loadability pose difficulties for complex mixtures. Recently, the analysis of hydrocarbon mixtures up to *n*-C_{130} has been achieved using a 300 × 0.3 mm i.d. column packed with 5 μm C_{18} bonded silica. The true boiling points and retention times of *n*-alkanes, alkylbenzenes, PAH and thiophenes have been correlated and it has been found that the retention time differences do not exceed 1 min for chemically different solutes with similar boiling points.

The effect of C_1 to C_{18} alkyl groups bonded to silica has been investigated and the oligomer peak resolution obtained with packed capillary columns approaches that obtained with open tubular columns. **Figure 6** shows the SFC chromatogram of the SD calibration standard on a packed capillary hexylsilyl (C_6) column. The SD data from SFC correlated well with those obtained by GC. Higher-molecular-weight hydrocarbons can easily be eluted at operating pressures below 415 bar (density of CO_2 mobile phase approximately 0.71 g L^{-1}). At this maximum pressure a column packed with hexyl (C_6) bonded silica elutes hydrocarbons boiling at more than 756°C,

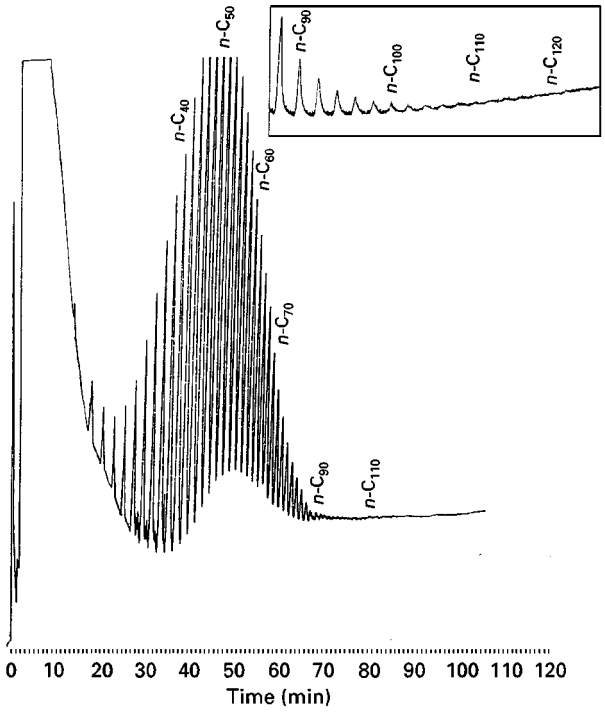

Figure 6 Simulated distillation chromatogram of Polywax 655 on a 0.25 m × 250 μm i.d. column packed with Waters Spherisorb S5W C_6. Conditions: linear pressure programming from 100 to 415 bar (hold for 60 min) at 3.5 bar min^{-1}, FID detection. (Courtesy of the University of Leeds.)

whereas a column packed with octadecyl silanol (ODS-2) (C_{18}; **Figure 7**) is more retentive and only elutes hydrocarbons boiling up to 686°C. Hydrocarbons of even higher boiling points can be eluted if the column length is changed. The significant deviation between the retention times of aromatics and straight chain alkanes of apparently similar boiling points which occurs when SD is performed by GC may be reduced using open tubular SFC and further minimized when packed capillary columns are used, especially for aromatic compounds with three or more rings. However, comparisons may not be valid in view of the discrepancies between published values of PAH boiling points.

Analysis of Zinc Dialkyldithiophosphate in Lubricating Oil

Zinc dialkyldithiophosphates (ZDDPs) are used in lubricating oils as extreme-pressure anti-wear additives. It is possible to analyse ZDDPs using SFC; if the sample is in a lubricating oil matrix then it is essential that a phosphorus-specific detector is used, i.e. nitrogen phosphorous detector (NPD) (**Figure 8**). This removes the interference from the base oil which is obtained if FID detection is used. **Figure 8** also shows that it is possible to determine other lubricating oil additives e.g. Irgafos 168.

Conclusions

The analysis of high-boiling hydrocarbon mixtures has historically been difficult due to their complex nature. Because of the favourable properties of supercritical fluids – low viscosity, low density and high diffusivity – SFC has found many applications in this area. The technique is becoming increasingly popular in the petroleum industry, especially for group-type separation and for simulated distillation. The main

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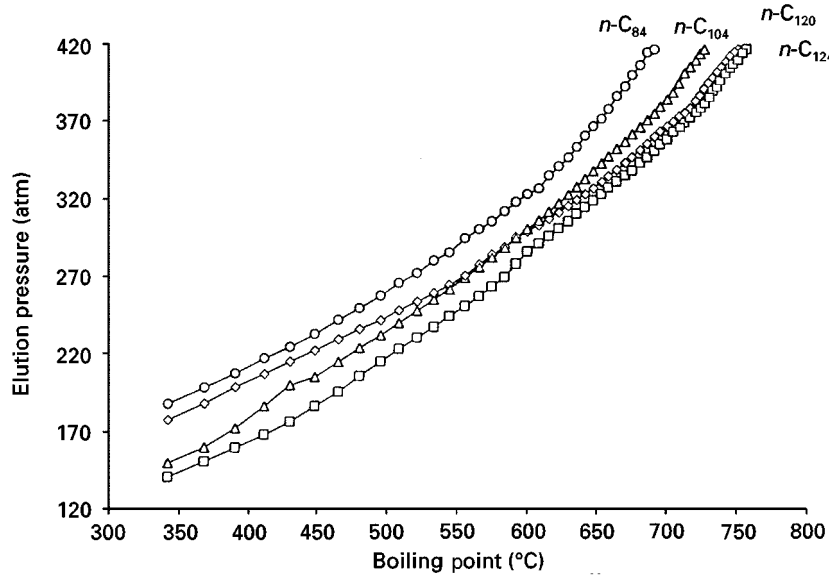

Figure 7 Graph of elution pressure versus the boiling point for packed columns. Diamonds, methyl (C_1); squares, hexyl (C_6); triangles, octyl (C_8); circles, ODS2 (C_{18}). (Courtesy of the University of Leeds.)

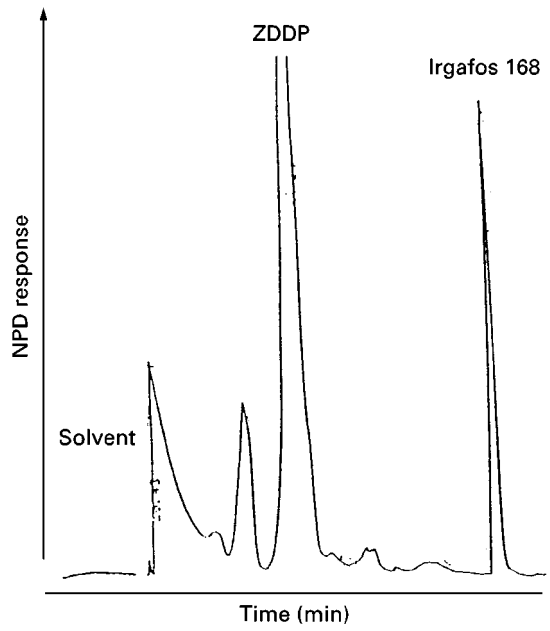

Figure 8 Chromatogram of used lubricating oil. Conditions: Diol packed column, temperature 50°C; ramp rate of 3 bar min^{-1}, NPD detection. (Courtesy of the University of Leeds.)

advantages of SFC are its speed of analysis and improved column efficiency when compared to liquid chromatography. The lower column temperature than needed for GC allows the analysis of higher-molecular-weight mixtures with carbon numbers up to C_{120} and beyond.

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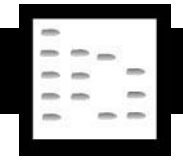
FULLERENES: LIQUID CHROMATOGRAPHY



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Introduction

Since the first separation in 1990 of C_{60} and C_{70} using column liquid chromatography (LC), this technique has played a very important role in fullerene chemistry. LC has allowed macroscopic quantities of fullerenes (particularly C_{60}) to be isolated and purified from the processing products. Obtaining sufficient amounts of pure fullerenes has been crucial both for determining physical and chemical properties in order to investigate practical applications of this new variety of carbon, and for developing a chemistry of these spherical and polyfunctional carbon molecules. A very rich chemistry has been developed in less than a decade based mainly on C_{60}, and to a lesser extent on higher fullerenes. The starting reactives for this chemistry have been the compounds previously isolated by LC. It is now possible to bind covalently many types of compounds to the fullerene molecule.

LC is currently the method of choice for the separation, isolation and purification of fullerenes. Progress in fullerene chemistry therefore depends on the development of improved chromatographic methods, i.e. those with the highest efficacy and best resolution between the different components of fullerene mixtures, at both analytical and preparative scales, and at the lowest cost.

Contribution of LC in the Field of Fullerene Production

In early 1990, the design of LC methods was mostly geared towards isolating the most abundant